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AN EPITHELIAL CELL-FREE PREPARATION OF THE MOTOR NERVE NET OF *CYANEA* (COELENTERATA; SCYPHOZOA)

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Abstract

Neurons of the motor nerve net of the jellyfish *Cyanea* lie between the cell bodies and muscle tails of myoepithelial cells. These myoepithelial cells are connected by septate desmosomes and, consequently, constitute a diffusion barrier and limit access to the neurons. Two techniques have been developed to remove the myoepithelial cells. One employs osmotic shock, the other, oxidation of the surface of the myoepithelial cells. The neurons remain in place and attach to the underlying mesoglea. Individual neurons retain their normal appearance and, when bathed in a saline that matches the free-ion content of the mesoglea, they retain their resting potentials and produce normal action potentials. Furthermore, the synapses continue to function normally. By supplementing the saline, these preparations can be maintained in good condition for several days. Under these culture conditions, neurons damaged during isolation produce growth cones and appear to form connections with other neurons.

Introduction

The motor nerve net of the jellyfish *Cyanea capillata* is a network of bipolar neurons that transmits action potentials from the marginal ganglia to the swimming musculature (Anderson and Schwab, 1981). The neurons are connected by symmetrical chemical synapses (Anderson and Schwab, 1981), implying that conduction across the synapse is functionally non-polarized. Although the cells are small, the cell bodies are large enough (10–20 μm) to allow intracellular recordings. Estimates of the cable constants of the neurons and the arrangement of the synapses indicate that an electrode in the cell body of a neuron will record all that occurs at the terminal (Anderson and Schwab, 1983). Consequently, this preparation can provide information about the physiology of chemical synapses in this animal and, because of the physiological conventionality of these neurons (Anderson and Schwab, 1983), synapses in general.

The preparation used is the peri-rhopalial tissue (Anderson and Schwab, 1981). Here, neurons of the motor nerve net lie between the cell bodies and muscle tails of the myoepithelium that forms the ectoderm of the peri-rhopalial tissue (Anderson and Schwab, 1981). At their outer boundaries these myoepithelial cells are joined by septate desmosomes (Anderson and Schwab, 1981) suggesting they present a diffusion barrier for drugs and putative transmitters, as well as physically limit access to the neurons and their synapses. For these reasons, it would be useful to be able to remove these overlying cells. Two techniques for doing so have been developed and are described here. The first employs a combination of osmotic shock and Ca^{++}/Mg^{++}-free sea water, while the second relies on the oxidation of the surface of the myoepithelial

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cells by sodium hypochlorite. Both techniques were successful although the latter proved to be more reliable and useful.

Early studies with the exposed neurons revealed that their resting potentials and excitability were altered when they were bathed in sea water, the usual saline for the earlier experiments with unexposed neurons. This observation prompted an investigation into the true ionic environment of the neurons, since bathing the exposed cells in sea water might, on its own, account for their altered responses. There are no obvious diffusion barriers between the neurons and mesoglea of the animal (Anderson and Schwab, 1981). Assuming that the free ion content of the mesoglea would be similar, if not identical to that of the extracellular fluid, the mesoglea was used as the source of fluid for ion analysis. Exposed neurons bathed in the resulting saline had normal resting potentials and excitability. Furthermore, by supplementing the saline these preparations could be maintained in good condition for several days.

Materials and Methods

Ion analysis

Specimens of *Cyanea capillata* with bell diameters of 10 cm or greater were collected in the vicinity of the C. V. Whitney Laboratory. Shortly after collection, an animal was removed from the holding tank and its tentacles and oral veils removed. The central part of the bell was excised and cut into 1 to 2 cm cubes and adhering tissue and epithelial layers removed. The cleaned, now completely transparent, pieces of mesoglea were rinsed briefly in distilled water, blotted dry on filter paper, and placed in an ultra-filtration cell (Amicon 2100 CF 50), which was centrifuged for 15 min at 2500 rpm. This procedure produced approximately 2.5 ml of fluid per animal. The fluid from each animal was analyzed for osmotic strength (Wescor 5100B) and then divided into several separate vials and frozen.

Sodium and potassium concentrations were measured by flame photometry (Instrumentation Laboratory 443), calcium and magnesium concentrations by atomic absorption (Perkin-Elmer 2380), and chloride ion concentration by titration. For sulfate measurements, the fluid samples were first acid hydrolyzed to denature residual protein and then total sulfate was measured using $^{123}BaSO_4$ precipitation (Renfro and Dickman, 1980). In each case, fluid from three vials was analyzed for each animal and the final concentration calculated as the average of 5 animals (Table I).

The saline developed from this study was prepared according to the method of Wilkens (1970). Since the osmotic strength of the fluid from the mesoglea was found to be within the range of ambient sea water, all stock solutions were prepared isos-

Table I

The concentrations (mM) of ions in fluid from the mesoglea of Cyanea

Animal #	Na^+	K^+	Ca^{++}	Mg^{++}	Cl^-	SO_4^{--}
Cy 1	397.4	16.5	9.8	31.0	575.7	5.28
Cy 2	409.5	14.3	9.3	29.2	586.7	3.74
Cy 3	377.0	13.0	9.5	27.6	535.3	3.34
Cy 4	389.4	11.3	9.4	29.4	553.7	7.19
Cy 5	374.3	11.8	9.4	27.9	513.3	8.69
Mean	389.5	13.4	9.5	20.0	552.9	5.65
S.E.M.	6.52	0.94	0.09	0.61	13.31	1.02

motically with ambient sea water. The final concentrations (m*M*) of the various salts in the saline were: NaCl, 390; KCl, 13.4; $CaCl_2$, 9.5; $MgCl_2$, 24; $MgSO_4$, 5; choline chloride, 41.5; $NaHCO_3$, 2.7. Choline chloride was added to elevate the chloride concentration to that measured by ion analysis. Ca^{++}/Mg^{++}-free saline was prepared by adding an equivalent amount of NaCl. Na^+-free saline was prepared using Tris-Cl. The pH of homogenized mesoglea was 7.4 and the pH of all salines was adjusted to this value. For long-term culture, the saline was supplemented with 1 $mg \cdot ml^{-1}$ glucose (Sigma), 2% Fetal Bovine Serum (GIBCO), and 1% penstrep with fungizone (GIBCO). No attempt was made to use sterile procedures during the production or maintenance of the preparations.

Electrophysiological techniques

The recording techniques employed here have been described in detail elsewhere (Anderson and Schwab, 1981; 1983). Briefly, pieces of peri-rhopalial tissue (Anderson and Schwab, 1981) were removed from the animal and pinned with cactus spines (*Opuntia* sp.) to a layer of Sylgard (Dow Corning) on the bottom of a plastic petri dish. When such preparations were examined using Modulation Contrast Optics, neurons of the motor nerve net were seen very clearly (Fig. 1A). This basic procedure was followed both for intact preparations and as the first step in the preparation of exposed cells, although, with the latter, the adjoining muscle bands were not removed. Preparations of exposed cells were bathed in *Cyanea* saline. Tetrodotoxin (TTX), saxitoxin (STX) and Ni^{++} ($NiCl_2 \cdot 6H_2O$) were added directly to the saline as needed.

For intracellular recordings cells were impaled with 3 *M* KCl-filled microelectrodes pulled from either thin-walled glass (Supertip, Frederick Haer) (10–20 MΩ impedance) or conventional thicker-walled capillary glass (WP Instruments) (40–60 MΩ). Action potentials were evoked by current injected through the recording electrode. Injected current was measured with a virtual ground circuit in the ground return circuit; a high-frequency filter in this circuit tended to round-off the square current pulses (see Fig. 3A). Signals were amplified using conventional capacity-compensated amplifiers (Dagan Instruments, Getting Instruments) and displayed on a digital oscilloscope (Nicolet Instruments). Hard copies of stored records were produced using a Houston Instruments 100 X-Y plotter.

Removal of the epithelium

Several procedures have been used, with various results, to expose the neurons. No attempt was made to use enzymatic techniques since these might produce subtle changes that could escape detection. Mechanical abrasion of the overlying myoepithelial cells with a microelectrode held in a micro-manipulator was effective in exposing the neurons, but was extremely time consuming.

The most effective and reliable techniques were based either on brief osmotic shock or brief oxidation of the myoepithelial cell surface. The former technique was used because the myoepithelial cells all contain a large vacuole that occupies most of their volume (Anderson and Schwab, 1981). It was felt that if these cells could be damaged selectively, by bursting their vacuoles, the muscle tails might contract and release the cells. This procedure worked well, but better results were obtained by subsequent treatment with Ca^{++}/Mg^{++}-free saline that contained 10 m*M* EDTA.

When these two treatments were combined the results were extremely good, but also extremely variable. Sometimes prolonged exposure (5 min or more) to 20% sea water, or even distilled water was necessary, while with other preparations 30 seconds in low strength sea water proved too long, and when the solutions were changed the

entire ectoderm would float free. The same applied to the Ca^{++}/Mg^{++}-free treatments. Even in those preparations which were judged to have been appropriately treated, the ectoderm would be released from the mesoglea in a single sheet if subjected to excessive mechanical agitation. For this reason, mechanical disturbance was minimized; solutions were changed by pipette if necessary and the preparations left undisturbed when possible. Because of the variability encountered in this work, it is difficult to give a particular recipe for the most effective protocol. However, the basic procedure can be summarized as brief osmotic shock in 20% sea water until vacuole bursting is first observed, followed with one rinse by Ca^{++}/Mg^{++}-free saline with 10 m*M* EDTA. The preparation must be carefully examined during this latter treatment and at the first appearance of epithelial cell-free regions, the solution replaced with the final solution (supplemented *Cyanea* saline), with minimal disturbance. At this stage, the preparations were transferred to a refrigerator (9°C) for 12–24 hours, after which time the released epithelial cells would have formed a thin mucous-rich film over the entire surface. This layer would be removed with a pipette, leaving the neurons attached to the mesoglea.

For the second procedure, preparations of peri-rhopalial tissue were pinned out in the same manner and then immersed in a 0.05% solution of sodium hypochlorite (1:100 dilution of domestic bleach) for 1–5 seconds. This solution was poured off and the preparation immediately rinsed 5 times in saline and set aside, once again with minimal mechanical disturbance. After 2–3 hours the debris could be removed with a pipette, exposing the neurons. At this point the preparations were bathed in supplemented saline and kept at 9°C until needed.

Electron microscopy

Preparations of exposed neurons were fixed in 2.5% gluteraldehyde in 0.4 *M* Millonig's phosphate buffer and post-fixed in 1% phosphate buffered osmium. For ease of handling the pieces of fixed tissue were transferred to small pieces of Sylgard (Dow Corning, Michigan) and pinned in place using cactus spines (*Opuntia* sp.), before being dehydrated with ethanol, critically-point dried, and coated with gold/palladium. The tissue was examined using a JSM 35C operated at 15 kV.

Results

The procedures used to expose the neurons had a wide range of effects, and the results were often variable. The osmotic technique sometimes produced preparations in which most, if not all, of the nerve net was exposed with only small amounts of epithelium remaining (Fig. 1B, C, E–G); on other occasions, the epithelium was seemingly unaffected by the procedure, even though the epithelial cells appeared to have been damaged and dislodged during EDTA treatment. Finally, with some preparations the entire epithelium disappeared with only a few neurons remaining (Fig. 1D, H); the remainder of the preparation was completely clear. This variability was due, in part, to differences between animals. If pieces of peri-rhopalial tissue from different animals were pinned in the same dish, and thereby given identical treatment, the results for the two preparations were often quite different.

With the bleach treatment, too, the results were variable, but here the epithelium was almost always immediately irreversibly damaged; the variability occurred when the resulting debris was removed. With some preparations the neurons remained attached to the mesoglea and the debris could be removed easily; with others, the debris could not be removed without taking the neurons too. Nevertheless, this technique was much more reliable and convenient. The epithelial cells could be removed

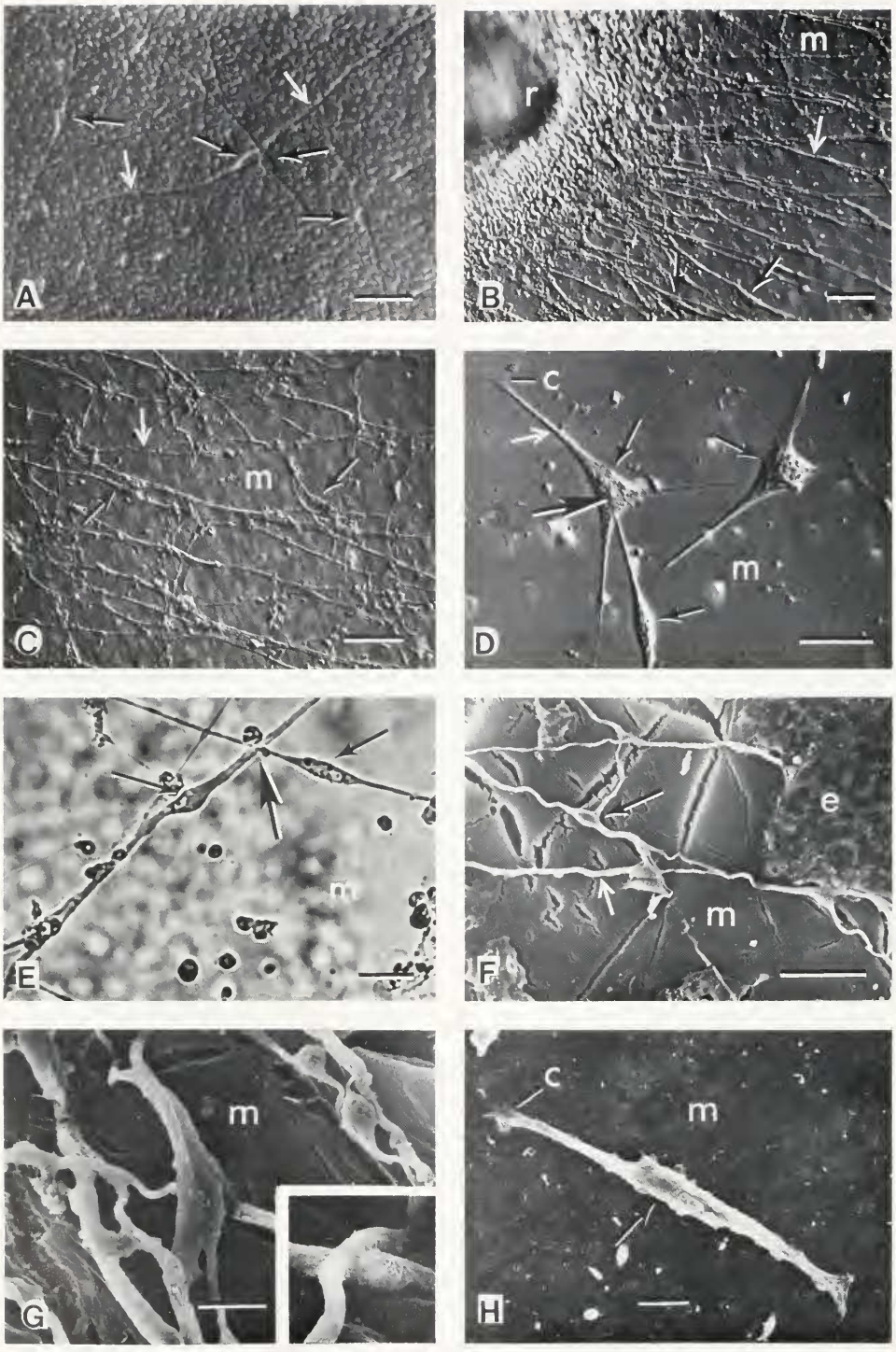

FIGURE 1. (A) A modulation contrast micrograph of the peri-rhopalial tissue of *Cyanea*. The cell bodies (black arrows) and axons (white arrows) of neurons of the motor nerve net are clearly visible. The cobblestone appearance of the bulk of the tissue is due to the vacuoles in the myoepithelial cells whose cell bodies cover the neurons. Bar = 50 μm. (B) A low-power modulation contrast micrograph of a portion of an epithelial cell-free preparation of the peri-rhopalial tissue immediately adjacent to the rhopalial (r).

more rapidly with the bleach treatment (2–3 hours as opposed to 12 hours), and subjective assessments of the end points of the stages in the osmotic protocol were avoided.

In preparations with a low neuron density, some cells were invariably found close to the muscle bands and in a fan-like array emerging from the rhopalium (Fig. 1B). This distribution may reflect the additional mechanical support given the neurons by these structures. If all muscle were removed from the peri-rhopalial tissue at the outset of the treatment, the stripping process occurred as described, but the preparation would later shrink and pull free from the cactus spines. For this reason, the usual preparation was one in which the adjoining radial and circular muscle bands were left intact. These preparations were less susceptible to shrinkage, although their marginal ends, which lacked muscle, would often pull away from the pins.

Morphology of exposed neurons

For the most part, the exposed cells retained their characteristic bipolar shape (Anderson and Schwab, 1981). This was particularly so for newly prepared preparations in which the density of neurons remained high (Fig. 1B, C). With such preparations individual neurons could be traced over considerable distances through several synapses. The dimensions of the neurons were no different from those of cells in intact preparations, although even with the cells so visible, it was not possible to determine the lengths of the longer fibers; the criss-crossing and merging of axons made it difficult to trace any one process for any distance. When preparations were maintained in supplemented saline for any length of time the dimensions and appearance of the neurons did change. Most noticeably, the cells flattened out onto the mesoglea, and as a result appeared far larger (Fig. 1D); many cells produced supernumerary processes (Fig. 1D).

In many exposed neurons, intracellular organelles, such as the nucleus, nucleolus, and a variety of vesicular structures, could be seen clearly (Fig. 1D, E) and movement of the organelles often could be discerned. Scanning electron microscopy of the exposed neurons revealed that their surfaces were, for the most part, clear of adhering material (Fig. 1F–H) and individual axons could be traced over considerable distances.

In preparations in which only a few neurons remained, the axons of the exposed cells were usually very short; the total length of the cell could be as little as 150 μm,

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The motor nerve net neurons remain in place and lie on the surface of the transparent mesoglea (m). Individual nerve cell bodies (black arrows) and their axons (white arrows) are visible. Bar = 100 μm. (C) A low-power modulation contrast micrograph of exposed neurons. Individual axons (white arrow) and cell bodies (black arrows) are visible and processes of the cells cross one another to form a complex two-dimensional nerve net: m—mesoglea. Bar = 100 μm. (D) A modulation contrast micrograph of neurons in a preparation in which the density of neurons was low. This preparation had been maintained for 1–2 days as evidenced by the flattening of the neurons, the formation of growth cones (c), and the unusually high frequency of tripolar neurons. The processes from the neurons make contact with one another (large arrows) at discrete points rather than in the normal *en passant* fashion. Numerous small vesicular structures can be seen in these cells. Many such organelles were motile. Bar = 50 μm. (E) A phase micrograph of a presumed synapse (large arrow) between two neurons in an epithelial cell-free preparation. The neurons (small arrow) are obvious against the clear mesoglea (m). Bar = 25 μm. (F) A low-power scanning electron micrograph of an epithelial cell-free preparation. At the edge of the exposed area, the surface of the epithelial cell layer (e) is visible. The axons (white arrows) and cell bodies (black arrows) of exposed neurons are visible against the mesoglea (m) which had fractured during drying. Bar = 50 μm. (G) A higher-power scanning micrograph of exposed neurons. The axons of the neurons criss-cross and parallel one another to form a complex series of connections: m—mesoglea. Bar = 20 μm. Inset. A presumed synapse between two neurons. The axons are very closely opposed and no gap is visible between them. (H) A scanning micrograph of a single exposed neuron. The soma is visible as a swelling (arrow) but the axons are very short. At one end the cut axon forms a growth cone (c): m—mesoglea. Bar = 20 μm.

as opposed to the 0.5–1.0 cm that has been reported for cells in other medusae (Horridge, 1954). When these preparations were maintained, the neurons developed additional processes as already described, and the cut ends of the axons produced growth cones (Figs. 1D, H; 2) which migrated across the mesoglea. Fine filopodia (0.13–0.4 μm diameter) projected from the expanded axon tip. The lengths of the filopodia were variable, but several could be traced for 30–40 μm. The cone itself could be up ~20 μm in diameter and much of this consisted of a thin sheet or lamellipodium.

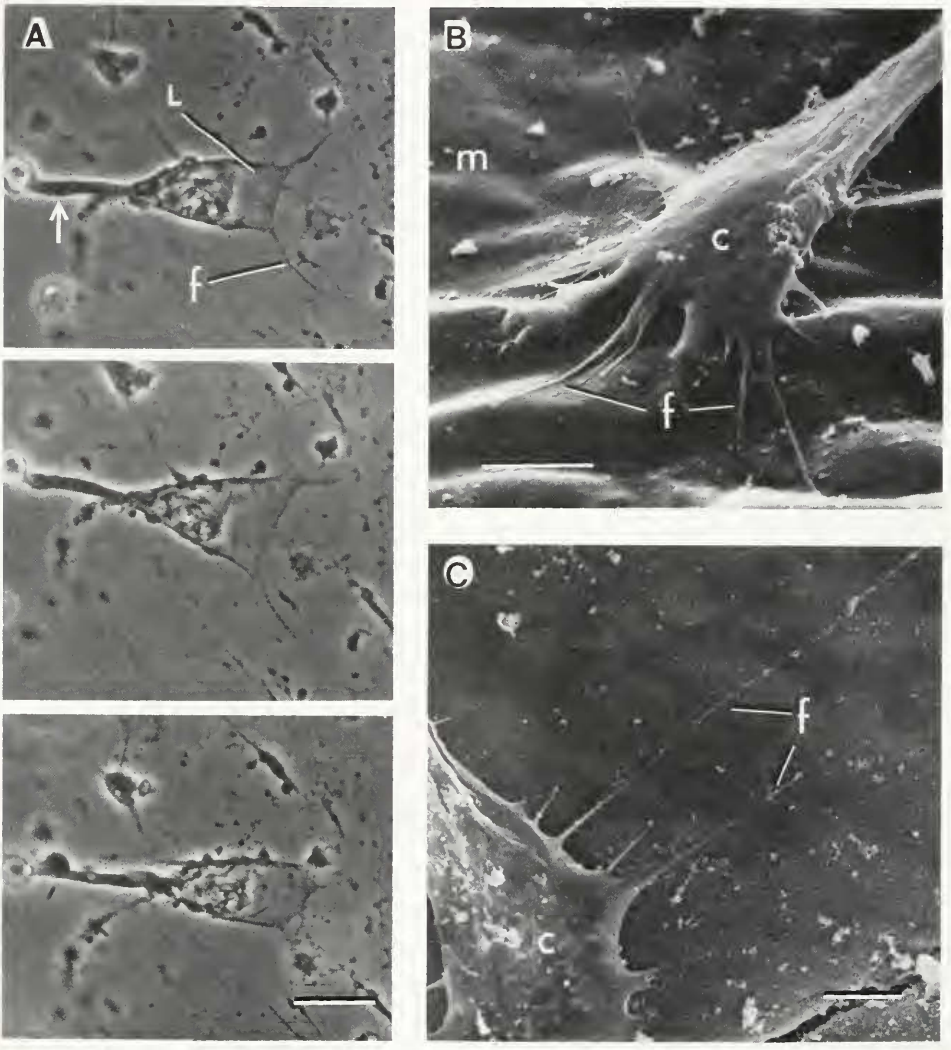

FIGURE 2. (A) Three phase micrographs of single growth cone taken at intervals of 8 and 17 minutes. The growth cone is an expansion at the end of the axon (white arrow) and consists of a broad lamelipodium (L) from which emerge numerous filopodia (f). Growth cones continually changed shape as they spread over the mesoglea. Such shape changes are obvious here. Bar = 20 μm. (B & C) Scanning electron micrographs of growth cones (c) on exposed neurons. Filopodia (f) emerge from the expanded cone and extend for long distances over the mesoglea (m). Bar = 5 μm.

The cones were extremely active: they changed their shape continuously and rapidly as the filopodia extended and retracted from the cone. Figure 2A shows three micrographs of the same cone taken at intervals of 8 and 17 minutes. The overall shape of the cone changed throughout the series, as did its position relative to other objects in the field of view. No attempt was made to quantify the rate of growth of the axon or filopodia. With preparations that contained fairly large numbers of neurons, the growing axons did appear to form contacts with other neurons (Fig. 1D) but, at this time, it is not clear if those connections were functional synapses.

Intracellular recordings from exposed neurons

Intracellular recording from unexposed neurons is quite feasible, but fairly difficult since the cells are very small. The overlying epithelium may aid impalement by preventing lateral movements of the neurons, but when the epithelium is present a microelectrode can be used for only one attempted penetration. If that is unsuccessful, then the microelectrode has to be discarded. This need to constantly change the electrodes is obviously time-consuming. With exposed cells, the problems are greatly reduced. It is easier to impale the exposed cells, and a single microelectrode can be used for several successive cells.

When exposed cells were bathed in normal sea water or artificial sea water (ASW), their resting potentials were significantly different from those of cells covered by intact epithelium (*in situ* cells). The mean resting potential of *in situ* cells was -58 ± 2 mV (SEM; n = 40) (Anderson and Schwab, 1983), while that of exposed cells was -67.9 ± 0.9 mV (n = 26). Furthermore, it proved very difficult to evoke action potentials in exposed cells. Extracellular axonal stimulation, usually the most effective means of evoking action potentials in these cells, was successful only when high intensity stimuli were used, and usually the axon under the stimulating electrode was so damaged by the stimulus that further spikes could not be evoked without moving the stimulating electrode. Intracellular current injection, a technique frequently successful with *in situ* cells, was almost totally ineffective. It was these findings that prompted the search for a suitable saline for these neurons.

Exposed cells bathed in *Cyanea* saline had resting potentials in the range of -46 to -69 mV (mean = -58.5 ± 1.5; n = 16). These values are almost identical to those of *in situ* cells. Furthermore, their excitability remained unaffected. Representative action potentials from exposed cells are included in Figure 3. These spikes were evoked by intracellular current injection and they display much of the variability and complexity of spikes recorded from neurons in intact preparations (Anderson and Schwab, 1983). The mean overshoot of action potentials in exposed cells bathed in saline that contained 10 m*M* Ni^{++} to block the synapses was 38 mV $\pm$ 3.4 (SEM; n = 6). This compares favorably with the 33 mV overshoot for *in situ* cells under similar conditions. The hyperpolarizing undershoot in the exposed cell took up to 1850 ms to decay following a single action potential. Once again, this is consistent with results from *in situ* cells (Anderson and Schwab, 1983). Superimposed on the action potentials were small depolarizing potentials. These have been shown to be synaptic potentials (Anderson and Schwab, 1983). With some cells, these occurred up to 28 ms after the spike (Fig. 3B). This delay is far larger than that typically encountered with *in situ* neurons and may, in part, reflect damage. Alternatively, the increased delay may merely reflect the added time taken for an impulse to spread through the less dense nerve net, where pathways would be longer, and return to the recorded cell via the recurrent pathways inevitably present in a diffuse nerve net (Anderson and Schwab, 1983).

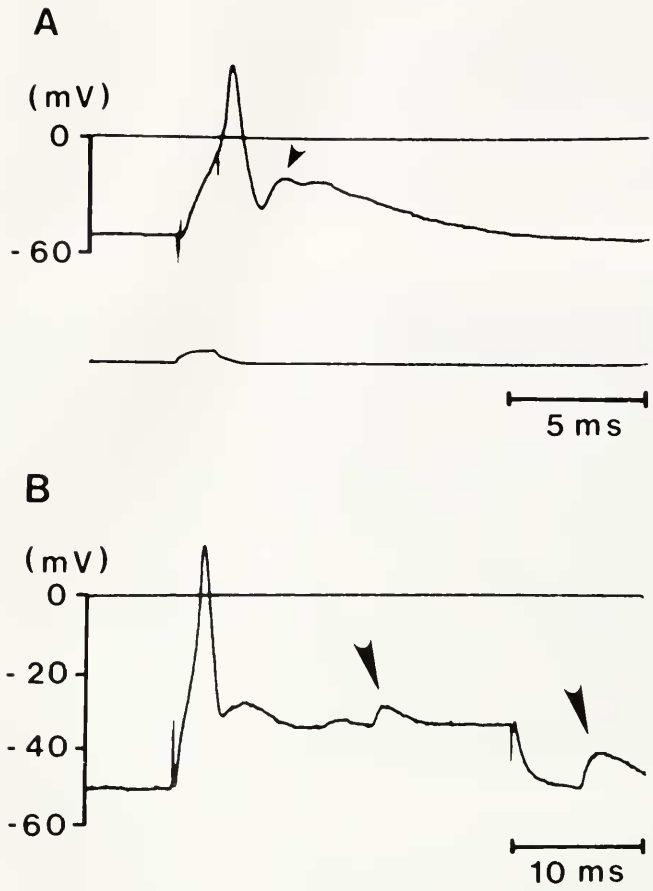

FIGURE 3. Action potentials recorded intracellularly from exposed neurons. (A) Upper trace: a single action potential evoked by a brief (2 ms) current pulse. Lower trace: current record. The action potential occurred when the current pulse had ended and was followed by a complex synaptic depolarization (arrow head). (B) A single action potential evoked by a longer current pulse. Individual synaptic potentials (arrowheads) are visible.

To further verify that the exposed cells were in good condition, they were current clamped, and I/V curves produced (Fig. 4). As with *in situ* cells, they displayed pronounced delayed rectification and, for hyperpolarizing pulses, had input impedances in the range 35–75 MΩ. This is consistent with data from *in situ* neurons (Anderson and Schwab, unpub.).

Preparations of exposed neurons maintained at 9°C in supplemented *Cyanea* saline remained active and functional for several days. When these preparations included swimming muscle, a stimulus to the center of the preparation would produce muscle contraction if there were an intact pathway to the muscle. This phenomenon occurred for up to 4 days post-exposure, and action potentials in cells maintained for this period contained synaptic potentials typical of those in freshly exposed or *in situ* cells.

One of the chief reasons for developing the exposed cell preparation was to remove the permeability barrier posed by the overlying epithelial cell bodies. Earlier (Anderson

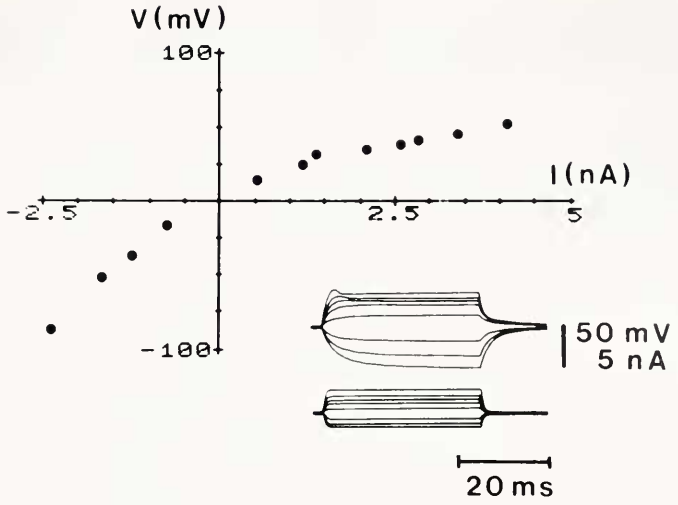

FIGURE 4. The current/voltage relationships of an exposed cell. Current clamp records from this cell are included as an inset. The exposed neurons have input impedances typical of those of unexposed neurons and display delayed rectification upon depolarization.

and Schwab, 1983) it was noted that the sodium channel blocker tetrodotoxin (TTX) (Narahashi *et al.,* 1964) was ineffective, even at high concentrations. It could be argued, however, that this observation was the result of impermeability, rather than true TTX insensitivity. However, exposed cells continued to produce normal action potentials after 24 hours in 1.25×10^{-5} *M* TTX. The cells were also insensitive to 10^{-6} *M* saxitoxin (STX), which had not been used previously with these or other coelenterate neurons. Since the effects of TTX and STX are similar if not identical (Narahashi, 1974) the absence of STX sensitivity is not surprising.

The amplitude of the action potential in intact preparations is dependent on the extracellular sodium concentration (Anderson and Schwab, 1983). However, the relationship between the two (88 mV/decade) was not that predicted by the Nernst equation, and at the time, it was thought that the discrepancy might be a consequence of ion pumps in the overlying epithelium. To test for this, these experiments were repeated using exposed neurons.

Preparations were bathed in *Cyanea* saline that contained either the normal amount of sodium or 50% sodium (Tris substituted). EPSPs, which confer amplitude and waveform variability to the spike, were blocked by the addition of 10 m*M* Ni^{++} to the medium. Action potentials were evoked by current injected through the microelectrode. The results confirmed the earlier finding that the spike is sodium dependent (Anderson and Schwab, 1983). The relationship between overshoot amplitude and extracellular sodium was 49.7 mV/decade. Calcium-free saline was not used here because of its potential for artificially depolarizing the neurons (Frankenheuser and Hodgkin, 1957), and although the Ca^{++} channels were blocked by Ni^{++} (Adams *et al.,* 1980), some Ca^{++} may have entered through the Na^{+} channels. This Ca^{++} entry could account for the discrepancy between the slope obtained here, and that predicted by the Nernst equation (58 mV/decade). Interestingly, the slope obtained here is similar to that for *in situ* cells in the presence of ouabain (47 mV/decade) (Anderson and Schwab, unpub.), confirming the earlier belief (Anderson and Schwab, 1983)

that the unusual relationship between spike overshoot in *in situ* cells and external Na^+ concentration can be explained by the action of transepithelial ion pumps.

Discussion

The neurons of the motor nerve net of *Cyanea* are normally covered by the cell bodies of the myoepithelial cells of the peri-rhopalial tissue ectoderm (Anderson and Schwab, 1981). While these cells do not obscure the neurons (Fig. 1A) nor prevent cell impalement, they form an obvious barrier that restricts physical access to the neurons and, more important, to their synapses. This barrier would limit the usefulness of this preparation by restricting access of putative transmitters applied to the synapses. Here we describe the results of a study designed to circumvent these problems by devising ways of removing the overlying cells.

Two techniques are described. Although their effects were variable, both were very successful, producing good preparations of exposed, clean neurons. Of the two, the sodium hypochlorite technique was by far the more convenient and reliable. It is, however, the one most open to criticism. Sodium hypochlorite is a powerful oxidizer and one which, even in very low concentrations, could damage the neurons. However, it was used here assuming that the septate desmosomes that connect the myoepithelial cells would protect the underlying neurons, at least temporarily, by preventing the diffusion of the hypochlorite into the extracellular spaces. The fact that the physiology of the exposed neurons was seemingly normal supports this belief.

Neurons in the exposed cell preparations were morphologically normal and, when damaged, produced growth cones which closely resembled growth cones of higher organisms (Landis, 1983). The cut axons grew and appeared to form connections with other cells (Fig. 1D). The exposed neurons lie on the mesoglea, an acellular matrix with some chemical similarities with the basement membranes of vertebrates (Barzansky and Lenhoff, 1974; Barzansky *et al.*, 1975). Mesoglea is known to be an excellent matrix for attachment and spreading of coelenterate cells (Day and Lenhoff, 1981). Those authors used *Hydra* mesoglea as a substrate for attachment of a variety of non-specified *Hydra* cell types and found that of the various alternative substrates tested, including collagen and other tissue culture substrates, mesoglea was the most effective. It is not surprising, therefore, to find that *Cyanea* mesoglea is an excellent substrate for neurons from this species. It should be noted, however, that in intact peri-rhopalial tissue the neurons are separated from the mesoglea by the muscle tails of the myoepithelial cells (Anderson and Schwab, 1981) and have to settle onto the mesoglea once the intervening muscle tails have been removed. The fact that there is initially this separation between the mesoglea and the neurons explains why the newly prepared exposed cell preparation is so susceptible to mechanical disturbance.

This ability for the neurons to grow and apparently reform connections is not surprising. Romanes (1885) noted that when the subumbrella surface of *Aurelia* was cut with a shallow incision extending across the entire subumbrella, coordination of the muscles on either side of the cut was lost initially, but redeveloped after as little as 4 hours. This observation was interpreted as evidence for regeneration, a likely interpretation in view of the findings presented here.

When exposed neurons were bathed in sea water, their resting potentials and excitability were altered; specifically, the resting potentials were significantly more negative and the cells were far less excitable. These changes can be explained by differences between the ionic composition of the sea water (or ASW) and that of the extracellular fluid (Table II). The K^+ concentration of the extracellular fluid is higher than that of sea water, so exposed cells bathed in sea water would be hyperpolarized

TABLE II

Concentration (mM) of ions in fluid from Cyanea *and artificial sea water (ASW)*

	Na^+	K^+	Ca^{++}	Mg^{++}	Cl^-	SO_4^{--}
Cyanea c.	389.5	13.4	9.5	28.8	552.9	5.65
Cyanea c.[1]	442.0	16.2	19.9	100.0	556.0	22.4
ASW[2]	462.3	9.72	9.86	51.2	506.12	27.0

[1] From Koizumi and Hosoi (1936).
[2] From Wilkens (1970).

by the reduced K^+ concentration. Similarly, the Mg^{++} concentration of the mesogleal fluid is significantly lower than that of sea water, so when exposed cells were bathed in sea water they would be in an abnormally high extracellular Mg^{++} concentration. In amphibians, when the extracellular concentrations of divalent cations such as Ca^{++} and Ni^{++} are increased, frog nerve becomes less excitable and greater levels of depolarization are needed to elicit a given response (Hille, 1968). A similar situation could apply here when Mg^{++} levels are increased.

The only other remarkable difference between the ion contents of the mesoglea and sea water concerns sulfate. The sulfate concentration of the mesoglea was invariably less than that of sea water and showed the most variation. Neither of these findings is surprising. Denton and Shaw (1961) and later Mackay (1969), demonstrated that many gelatinous marine organisms, including coelenterates, regulate their buoyancy by altering their internal sulfate concentrations. Lift is obtained by exclusion of sulfate and, presumably, negative buoyancy by its accumulation. The jellyfish used in this study were collected by dipping from the surface and such animals would, according to this model, have achieved this position in the water column by excluding sulfate. The variability in the sulfate levels can be explained by a similar argument, considering that some animals were collected at a slightly greater depth than others, and would presumably be neutrally buoyant, hence having higher sulfate levels.

With the exception of these differences, the ionic composition of the mesoglea is essentially the same as that determined by Wilkins (1970) for sea water and similar to that measured in an earlier study (Koizumi and Hosoi, 1936) (Table II). It should be noted that this latter study used an intact piece of exumbrella, so some intracellular ions also would have been included. This and the less exact procedures used could account for the slight discrepancies between our findings and theirs.

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